National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2384

Baking Chocolate

Standard Reference Material (SRM) 2384 is intended primarily for use in validating methods for determining proximates, fatty acids, calories, vitamins, elements, catechins, caffeine, theobromine, and acrylamide in baking chocolate and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 2384 consists of five 91 g (3.2 oz) individually wrapped bars of baking chocolate.

Certified Concentration Values: The certified concentration values of fat, selected fatty acids, calcium, iron, caffeine, theobromine, and catechins in SRM 2384 are provided in Tables 1 and 2. Values were derived from the combination of results provided by NIST and collaborating laboratories. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST [1]. The certified values in this material are the equally weighted means of the mean NIST result and the mean of the measurements made by collaborating laboratories; the associated uncertainties are expanded uncertainties at the 95 % level of confidence [2,3]. Values are reported on an as-received (not dry-mass) basis in mass fraction units [4].

Reference Concentration Values: Reference concentration values for additional proximates, fatty acids, elements, calories, total dietary fiber, and other analytes are provided in Tables 3 through 6. Reference values are noncertified values that are the best estimate of the true values based on available data; however, the values do not meet the NIST criteria for certification [1] and are provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Expiration of Value Assignment: The value assignment of this SRM is valid until **31 December 2008**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. Value assignment is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Value Assignment: NIST will monitor this SRM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by K.E. Sharpless of the NIST Analytical Chemistry Division and H.B. Chin, I-P. Ho, and D.W. Howell of the National Food Processors Association (NFPA, Dublin, CA and Washington, DC).

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

The support aspects involved with the issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert and C.S. Davis of the NIST Measurement Services Division.

Willie E. May, Chief
Analytical Chemistry Division

Robert L. Watters, Jr., Acting Chief
Measurement Services Division

Gaithersburg, MD 20899
Certificate Issue Date: 28 September 2004
See Certificate Revision History on Page 8

Analytical measurements at NIST were performed by J. Brown Thomas, B.C. Nelson, C.S. Phinney, B.J. Porter, K.E. Sharpless, J.R. Sieber, and L.J. Wood of the NIST Analytical Chemistry Division. Analyses for value assignment were also performed by the laboratories listed in Appendices A through D.

NOTICE AND WARNING TO USERS

Storage: Until required for use, the baking chocolate should be stored under refrigeration.

Warning: For laboratory use only. Not for human consumption.

INSTRUCTIONS FOR USE

Test portions for analysis may be melted or grated as described in AOAC Official Method 970.20 [5]. Test portions used for NIST analyses described below were: 5 g to 6 g for tocopherols; 1 g for fat, fatty acids, catechins, caffeine, theobromine, and theophylline; and 0.5 g for calcium, iron, and sodium.

PREPARATION AND ANALYSIS

Preparation: SRM 2384 is baking chocolate prepared from 100 % cocoa beans and taken from a single production lot.

NIST Analyses for Fat: Two sets of seven samples of chocolate were prepared for analysis by pressurized-fluid extraction. One-gram portions of grated chocolate were extracted into petroleum ether. Extracts were evaporated under nitrogen and then dried at 100 °C to constant mass, per AOAC Official Method 963.15, Fat in Cacao Products [5].

NIST Analyses for Fatty Acids: Fatty acids were measured in three sets of five samples of chocolate over a three-day period. Using pressurized-fluid extraction, fat was extracted into petroleum ether from approximately 1 g samples of grated chocolate. Methyl nonadecanoate was used as an internal standard. A two-step process employing methanolic sodium hydroxide and boron trifluoride was used to convert the fatty acids to their methyl esters (FAMEs). FAMEs were extracted into hexane and injected into a gas chromatograph employing flame ionization detection.

NIST Analyses for Calcium, Iron, and Sodium: Calcium, iron, and sodium were measured in five bars of chocolate. Five whole bars were melted in individual beakers, and two 0.5 g portions were taken from each bar and digested in nitric acid in a microwave oven. Digests were transferred to plastic bottles and diluted with the appropriate volume of 1.5 % (volume fraction) nitric acid. To correct for matrix effects caused by differences between samples and calibrants, the method of standard additions was used; spikes were added to one aliquot prepared from each 0.5 g test portion. Four measurements using inductively coupled plasma optical emission spectrometry (ICP-OES) were made and averaged for each sample and each spiked solution. Results were corrected for spike recoveries. The ICP-OES result for sodium was confirmed using X-ray fluorescence (XRF) spectrometry and the method of standard additions.

NIST Analyses for Tocopherols: δ-Tocopherol, γ- (plus β-) tocopherol, and α-tocopherol were measured using liquid chromatography (LC) in single squares taken from each of eight bars of chocolate over a five-day period. (The baking chocolate may contain β-tocopherol, but the chromatographic system described below is incapable of resolving β- and γ-tocopherol; the instrument was calibrated using only γ-tocopherol.) Samples of approximately 5 g to 6 g were melted and then saponified using potassium hydroxide. Analytes were extracted into a mixture of diethyl ether and hexane, which was subsequently evaporated, and the analytes were redissolved in a mixture of ethanol and ethyl acetate. Samples were injected onto a C_{18} column and analytes were eluted using a gradient of acetonitrile, methanol, and ethyl acetate. (Additional methodological detail is provided in reference 6.) Both a programmable UV/visible absorbance detector set to 292 nm for measurement of the tocopherols and 422 nm for measurement of *trans*-β-apo-10'-carotenal oxime (the internal standard) and a fluorescence detector (excitation wavelength of 295 nm, emission wavelength of 335 nm) were used for quantitation of the tocopherols.

NIST Analyses for Caffeine, Theobromine, and Theophylline: Caffeine, theobromine, and theophylline were measured in single 1 g test portions, taken from each of eight bars of chocolate over an eight-day period, using LC. The chocolate was melted, an internal standard (β-hydroxyethyltheophylline) was added, and fat was removed from the sample via four successive extractions into hexane. The defatted chocolate was dried under a stream of nitrogen.

Water was added, and the sample was placed in an ultrasonicating bath and then centrifuged. The supernatant was filtered twice. Samples were injected onto a C_{18} column and analytes were eluted using an isocratic mixture of acetonitrile, water, and acetic acid. Absorbance was measured at 274 nm.

NIST Analyses for Catechins: (+)-Catechin and (–)-epicatechin were measured in single 250 mg test portions taken from eight bars of chocolate on a single day. Approximately 1 g of chocolate was combined with an internal standard solution (tryptophan methyl ester hydrochloride), and the chocolate was melted. Fat was removed from the sample via three successive extractions into hexane. The defatted chocolate was dried under a stream of nitrogen. The dried powder was stirred, and approximately 250 mg were removed. Catechins from this aliquot were extracted into two portions of methanol via ultrasonication. The supernatants were filtered and combined. The extract was diluted with water. Samples were injected onto a C_{18} column, and analytes were eluted using a gradient of water and acetonitrile, both of which contained trifluoroacetic acid. Analytes were measured by liquid chromatography/mass spectrometry (LC/MS) with electrospray ionization using *m/z* of 291 for the catechins and *m/z* of 219 for the internal standard.

Analyses by Collaborating Laboratories: Data from several additional sources were used for certification of this material: two interlaboratory comparison exercises organized by the NFPA Food Industry Analytical Chemists Subcommittee (FIACS; 11 laboratories, listed in Appendix A, participated in the first exercise, in which proximates, vitamins, and elements were measured; 7 laboratories, listed in Appendix B, participated in the second exercise in which only acrylamide was measured); 17 laboratories participating in an exercise organized by the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) Acrylamide Working Group in which acrylamide was measured (Appendix C); and four laboratories participating in an exercise in which catechins were measured (Appendix D). Not every laboratory measured every analyte, and some data were excluded as outliers. This fact accounts for the discrepancy in the number of laboratories listed in the Appendices B and C and the number of laboratories participating in the associated exercises. The laboratories listed in Appendix A were asked to use AOAC methods or their equivalent, to make single measurements from each of two bars, and to report the analytical method that was used. The laboratories listed in Appendices B and C were asked to make single measurements from each of two bars. The laboratories listed in Appendix D were asked to use their usual methods to make single measurements of catechins in each of four bars. A summary of the methodological information and the number of laboratories using a particular analytical technique are provided in Appendix E. The methods used by NIST are included in this listing as well.

Homogeneity Assessment: The homogeneity of calcium, iron, sodium, caffeine, theobromine, theophylline, fatty acids, tocopherols, and catechins was assessed at NIST using the methods described above. A small but statistically significant heterogeneity was found for calcium, and an inhomogeneity component has been included in the expanded uncertainty for this analyte. All other analytes, including those for which homogeneity was not assessed, have been treated as homogeneous.

Value Assignment: The laboratories listed in Appendix A reported values for two to four analyses. The laboratories listed in Appendix B reported values for one to two analyses, and the laboratories listed in Appendix C reported values for two to three analyses. The laboratories listed in Appendix D reported values for four to eight analyses. The mean for each laboratory was determined from these values, and a grand mean was calculated. In cases where NIST also made measurements, this grand mean was averaged with the NIST mean to obtain the assigned value. In cases where NIST did not make measurements, the grand mean became the assigned value. In cases where only NIST measurements were made, the NIST mean became the assigned value.

Table 1. Certified Concentration Values for Fat and Selected Fatty Acids[a]

Constituent	Mass Fraction (%)
Fat (Extractable)	51.4 ± 1.1
Fat (Sum of Fatty Acids)[b]	50.3 ± 1.1

Constituent	Mass Fraction (%) As the Triglyceride	Mass Fraction (%) As the Fatty Acid
Tetradecanoic Acid (C14:0) (Myristic Acid)	0.080 ± 0.005	0.076 ± 0.005
Hexadecanoic Acid (C16:0) (Palmitic Acid)	13.06 ± 0.27	12.44 ± 0.26
(Z)-9-Hexadecenoic Acid (C16:1) (Palmitoleic Acid)	0.133 ± 0.007	0.127 ± 0.007
Octadecanoic Acid (C18:0) (Stearic Acid)	18.01 ± 0.40	17.24 ± 0.38
(Z)-9-Octadecenoic Acid (C18:1) (Oleic Acid)	16.44 ± 0.36	15.73 ± 0.35
(Z)-11-Octadecenoic Acid (C18:1) (Vaccenic Acid)	0.180 ± 0.018	0.172 ± 0.017
(Z,Z)-9,12-Octadecadienoic Acid (C18:2) (Linoleic Acid)	1.524 ± 0.048	1.458 ± 0.046
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3) (Linolenic Acid)	0.097 ± 0.006	0.093 ± 0.006
Eicosanoic Acid (C20:0) (Arachidic Acid)	0.521 ± 0.013	0.501 ± 0.012
Docosanoic Acid (C22:0) (Behenic Acid)	0.091 ± 0.006	0.088 ± 0.006
Tetracosanoic Acid (C24:0) (Lignoceric Acid)	0.050 ± 0.002	0.050 ± 0.002

[a] Each certified concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A and NIST. The uncertainty in the certified values, calculated according to the method described in the ISO and NIST Guides [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix E. Some fatty acid data from collaborating laboratories were excluded if (1) the laboratory's result for a particular fatty acid disagreed with the majority of the other laboratories' results and if that laboratory's mean result was more than three standard errors from the mean of the interlaboratory comparison exercise or (2) if the standard deviation of that laboratory's results for a particular fatty acid was an order of magnitude larger than any other laboratory's standard deviation for that fatty acid.

[b] Fat as the sum of the fatty acids represents the sum of quantified individual fatty acid peaks.

Table 2. Certified Concentration Values for Selected Additional Analytes[a]

Constituent	Mass Fraction (mg/kg)
Caffeine	1 060 ± 50
Theobromine	11 600 ± 1 100
Calcium	840 ± 74
Iron	132 ± 11
(+)-Catechin	245 ± 51
(–)-Epicatechin	1 220 ± 240
Catechin monomers[b]	1 490 ± 220

[a] Each certified concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by laboratories listed in Appendices A and D, and NIST. The uncertainty in the certified values, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix E.

[b] Sum of (+)-catechin and (−)-epicatechin. Sum was determined mathematically and analytically; see Appendix E.

Table 3. Reference Concentration Values for Proximates and Caloric Content[a]

Constituent	Mass Fraction (%)
Solids	98.37 ± 0.35
Ash	2.78 ± 0.11
Protein	13.18 ± 0.46
Carbohydrate (by difference)	32.4 ± 1.9
Total Dietary Fiber	14.5 ± 3.0
Calories[b]	(631.0 ± 9.3) kcal/100 g

[a] Each reference concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix E. (The certified values for fat are provided in Table 1.)

[b] The value for caloric content is the mean of individual caloric calculations from the laboratories listed in Appendix A. If the proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (as the sum of the fatty acids), protein, and carbohydrate, respectively, the mean caloric content is 635 kcal/100 g.

Table 4. Reference Concentration Values for Selected Fatty Acids[a]

Constituent	Mass Fraction (%) as the Triglyceride	Mass Fraction (%) as the Fatty Acid
Dodecanoic Acid (C12:0) (Lauric Acid)	0.022 ± 0.004	0.021 ± 0.004
Pentadecanoic Acid (C15:0)	0.018 ± 0.003	0.017 ± 0.003
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.115 ± 0.006	0.110 ± 0.006
9-Eicosenoic Acid (C20:1) (Gadoleic Acid)	0.023 ± 0.004	0.022 ± 0.004

[a] Each reference concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A. The uncertainty in the reference values, calculated according to the method described in the ISO Guide [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix E.

Table 5. Reference Concentration Values for Elements[a]

Element	Mass Fraction (mg/kg)
Copper	23.2 ± 1.2
Magnesium	2 570 ± 150
Manganese	20.3 ± 1.3
Phosphorus	3 330 ± 210
Potassium	8 200 ± 500
Sodium[b]	40 ± 2
Zinc	36.6 ± 1.7

[a] Each reference concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix E.

[b] The reference concentration value for sodium, expressed as a mass fraction on an as-received basis, is the mean result of analyses performed by NIST using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO and NIST Guides [2]. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence.

Table 6. Reference Concentration Values for Selected Vitamins and Other Analytes

Constituent	Mass Fraction (mg/kg)
δ-Tocopherol[a]	3.42 ± 0.47
γ-Tocopherol[a,b]	108.2 ± 1.9
α-Tocopherol[c]	7.2 ± 1.7
Vitamin B_2[d]	1.21 ± 0.16
Niacin[d]	12.1 ± 2.0
Theophylline[a]	151 ± 3
Total Procyanidins[e]	10 300 ± 1 100

Constituent	Mass Fraction (ng/g)
Acrylamide[f]	138 ± 17

[a] Reference values expressed as mass fractions for δ-tocopherol, γ-tocopherol, and theophylline are the means of results obtained by NIST using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO and NIST Guides [2]. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte.

[b] May include β-tocopherol.

[c] The reference value for α-tocopherol, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix A and NIST. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix E.

[d] Reference values for vitamin B_2 and niacin, expressed as a mass fraction on an as-received basis, are the weighted means of results provided by the laboratories listed in Appendix A. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix E.

[e] "Total procyanidins" represents the sum of (+)-catechin, (−)-epicatechin, and the dimer through the decamer of the procyanidin oligomers. This reference concentration value, expressed as a mass fraction on an as-received basis, is the weighted mean of results provided by the laboratories listed in Appendix D. The uncertainty in the reference value, calculated according to the method described in the ISO and NIST Guides [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence. Analytical methodology information is provided in Appendix E.

[f] The reference value for acrylamide is the weighted mean of results provided by the laboratories listed in Appendices B and C. The uncertainty in the reference values, calculated according to the method described in the ISO and NIST Guides [2], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix E.

REFERENCES

[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000).

[2] ISO; *Guide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st ed.; International Organization for Standardization: Geneva, Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994) available at http://physics.nist.gov/Pubs/.

[3] Levenson, M.L.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H-k.; Vangel, M.G.; Yen, J.H.; Zhang, N.F.; *An Approach to Combining Results from Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571-579 (2000).

[4] Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811 (1995).

[5] AOAC International; *Official Methods of Analysis of AOAC International*, 17th ed.; Horwitz, W., Ed.; Gaithersburg, MD (2000).

[6] Epler, K.S.; Ziegler, R.G.; Craft, N.E.; *Liquid Chromatographic Method for the Determination of Carotenoids, Retinoids, and Tocopherols in Human Serum and in Food*; J. Chromatogr. (Biomed. Applications), Vol. 619, pp. 37-48 (1993).

Certificate Revision History: 28 September 2004 (This revision reflections the addition of a reference value for acrylamide); 04 June 2003 (This revision reflects correction in 1) certified values for (+)-catechin, (–)-epicatechin, catechin monomers; 2) reference values for theophylline and total procyanidins; and 3) text of Appendix C-Other Analytes. Units for caffeine and theobromine given in mg/kg (were g/kg)); 22 March 2002 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet http://www.nist.gov/srm.

APPENDIX A

The laboratories listed below performed measurements that contributed to the certification of SRM 2384 Baking Chocolate.

Covance Laboratories; Madison, WI, USA
Dionex Corporation; Salt Lake City, UT, USA*
General Mills, Inc.; Minneapolis, MN, USA
Hormel Foods Corporation; Austin, MN, USA
Kraft Foods; Glenview, IL, USA
Nabisco, Inc.; East Hanover, NJ, USA
Nestlé USA; Dublin, OH, USA
Novartis Nutrition Corporation; St. Louis Park, MN, USA
Pillsbury; St. Paul, MN, USA
Ralston Purina Company; St. Louis, MO, USA
U.S. Department of Agriculture, Food Composition Laboratory; Beltsville, MD, USA
Woodson-Tenent Laboratories; Memphis, TN, USA

*Not an NFPA FIACS laboratory.

APPENDIX B

The NFPA FIACS laboratories listed below performed measurements that contributed to the value assignment of acrylamide in SRM 2384 Baking Chocolate.

Covance Laboratories; Madison, WI, USA
Eurofins Scientific; Memphis, TN, USA
General Mills, Inc.; Minneappolis, MN, USA
Health Canada; Ottawa, ON, Canada*
Kraft Foods; Glenview, IL, USA (samples analyzed by Silliker Laboratories)
National Food Processors Association;Washington, DC, USA

*Not an NFPA FIACS laboratory.

APPENDIX C

The JIFSAN Acrylamide Working Group laboratories listed below performed measurements that contributed to the value assignment of acrylamide in SRM 2384 Baking Chocolate.

American Oil Chemists Society; Champaign, IL, USA
Covance Laboratories; Madison, WI, USA
Eurofins Scientific; Memphis, TN, USA
Federal Institute for Risk Assessment (BfR); Berlin, Germany
Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition; College Park, MD, USA
FDA, Office of Regulatory Affairs; Lenexa, KS, USA
Food Research Institute, University of Wisconsin; Madison, WI, USA
General Mills, Inc.; Minneappolis, MN, USA
Health Canada; Ottawa, ON, Canada
Joint Research Centre, IRMM; Geel, Belgium
Livsmedelsverket (National Food Administration); Helsinki, Finland
National Food Processors Association; Washington, DC, USA
Nestlé; Lausanne, Switzerland
Procter and Gamble; Cincinatti, OH, USA
Swiss Quality Testing Services; Dietikon, Switzerland
The National Food Laboratory; Dublin, CA, USA

APPENDIX D

The laboratories listed below performed catechin measurements that contributed to the certification of SRM 2384 Baking Chocolate.

European Commission - DG Joint Research Centre; Ispra, Italy
Antioxidant Research Group, King's College; London, UK
M&M/Mars, Inc.; Hackettstown, NJ, USA
U.S. Department of Agriculture; Little Rock, AR, USA

APPENDIX E

The methodological information reported by laboratories whose results were used for value assignments is summarized below. The number of laboratories using a particular method is provided in parentheses.

Proximates, Fatty Acids, Total Dietary Fiber, and Calories

Solids	Moisture determined by mass loss after oven-drying: Forced-air oven (5) Vacuum oven (5) Convection oven (1)
Ash	Mass loss after ignition in muffle furnace (10)
Extractable Fat	Acid digestion, ether extraction (8) Alkali pretreatment, diethyl ether/petroleum ether extraction (1) Soxhlet extraction (1) Pressurized-fluid extraction (NIST)
Fatty Acids	Hydrolysis followed by gas chromatography (GC; 10 + NIST)
Nitrogen	Kjeldahl (5) Thermal conductivity (2) Pyrolysis, GC (1) Combustion (2)
Protein	Calculated; a factor of 6.25 was used to calculate protein from nitrogen results
Carbohydrate	Calculated; [solids − (protein + fat + ash)]
Total Dietary Fiber	Enzymatic – gravimetry (6)
Calories	Calculated; [9(fat) + 4(protein) + 4(carbohydrate)]

Vitamins

α-Tocopherol	Saponification – reversed-phase liquid chromatography (RPLC) - absorbance detection (3 + NIST) Saponification – RPLC - fluorescence detection (NIST) Saponification – normal-phase liquid chromatography (NPLC) - absorbance detection (1) Saponification – NPLC - fluorescence detection (2) Enzymatic digestion – RPLC - absorbance detection (1) Enzymatic digestion – NPLC - absorbance detection (1)
δ-Tocopherol	Saponification – RPLC - fluorescence detection (NIST)

γ-Tocopherol	Saponification – RPLC - absorbance detection (NIST) Saponification – RPLC - fluorescence detection (NIST)
Total Vitamin B_2	Microbiological (1) Digestion – fluorescence detection (2) Extraction – RPLC - fluorescence detection (3)
Niacin	Microbiological (6)

Elements

Calcium	Flame atomic absorption spectrometry (1) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (9 + NIST)
Copper	Flame atomic absorption spectrometry (2) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (8)
Iron	Flame atomic absorption spectrometry (2) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (8 + NIST)
Magnesium	Flame atomic absorption spectrometry (1) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (9)
Manganese	Flame atomic absorption spectrometry (2) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (7)
Phosphorus	Absorption spectrophotometry (1) Flame atomic absorption spectrometry (1) Inductively coupled plasma optical emission spectrometry (9)
Potassium	Flame atomic absorption spectrometry (2) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (8)
Sodium	Inductively coupled plasma optical emission spectrometry (NIST)
Zinc	Flame atomic absorption spectrometry (2) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma optical emission spectrometry (8)

Other Analytes

Caffeine and Theobromine	Extraction – RPLC - absorbance detection (6+NIST)
Theophylline	Extraction – RPLC - absorbance detection (NIST)
Catechins	Extraction – RPLC - mass spectrometry (MS) (1+NIST) Extraction – NPLC - fluorescence detection (l) Extraction – RPLC - absorbance detection (2)
Total Procyanidins	Extraction – NPLC - absorbance detection (1) Extraction – NPLC - mass spectrometry (1)
Catechin Monomers	Extraction – NPLC – absorbance detection (1)

	Mathematical summation (4+NIST)
Acrylamide	LC-mass spectrometry (MS; 1) LC- tandem mass spectrometry (MS/MS) (19) GC-MS (4) GC-MS/MS (1)